DOSE RESPONSE RELATIONSHIP OF RELAXIN ON THE PUBIC LIGAMENT OF THE MOUSE

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Summary: In the present work, the dose-response relationship of highly purified porcine relaxin has been examined on broadening of the pubic ligament in mice.

Using the method of Steinetz *et al.* with 7 days of oestriol priming, higher sensitivity of the pubic ligament was attained in mice with an original weight of 10 g than in those with a weight of 20 g. S-shaped curves were obtained; by increasing the relaxin doses after maximal effect had been reached a decreased action was observed.

With presomen priming, a relaxin effect was established after only 2 days instead of the usual 8 days but the action was markedly less than in the Steinetz test.

INTRODUCTION

In 1960 Steinetz and co-workers showed that the pubic ligament of the mouse is suitable for testing the action of relaxin ([1]). They illustrated a linear relationship between enlargement of the symphysis and relaxin over a wide range of dosage.

At the time the test was developed, the structure of relaxin was still unknown and there was no internationally recognized standard, a relaxin preparation of pig ovaries of 150 GPU/mg being used. In the meantime, it has been possible to clarify the protein structure of porcine relaxin which appears to be very similar to that of insulin with a molecular weight of 6,000 ([2]). In the present work an attempt has been made to test the dose-response action of this highly purified relaxin (3,000 GPU/mg) under various conditions.

MATERIAL AND METHODS

Female, virgin mice of the NMRI strain were used for the investigation. The animals were maintained under controlled conditions (light, humidity, nutrition) so that possible seasonal changes in hormonal levels were largely eliminated. Relaxin from the National Pituitary Agency, Baltimore, USA), dissolved in 0.2 ml of a 1% aqueous benzopurpurine solution, was administered subcutaneously and allowed to act for approximately 20 hours. Doses for the dose/response curves were increased geometrically with factor 3 i.e. 0.5, 1.5, 4.5, 13.5 and 40.5 GPU/mouse.

Since optimal relaxin activity is obtained only after pretreatment with oestrogen, the animals were primed either with oestradiol benzoate in peanut oil or with conjugated oestrogen (Presomen in aqueous solution).

For measurement of the pubic ligament, the animals were killed by cervical fracture, the pubic ligament freed along with parts of the pubic bone, placed on a slide and covered with a coverglass. Measurement of the ligament was carried out using a standard microscope with a calibrated ocular micrometer which allowed measurements accurate to 1/30 mm.

RESULTS

In the first part of the investigation, dose-response curves were made using two different weight classes of mice. Oestrogen priming consisted of subcutaneous injection of 5 μg oestradiol benzoate in 0.1 ml solution/mouse, 7 days being allowed for its action.

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Table 1. — *Results after treatment with relaxin following priming with 5 µg oestradiol benzoate.*

	Mice of initial weight 10 g				Mice of initial weight 20 g			
Relaxin dose in GPU	No. of animals	Breadth of pubic ligament in mm±SEM	Wt. of animals on day of measurement in g (max.+min.)	No. of unaltered uteri	No. of animals	Breadth of pubic ligament in mm±SEM	Wt. of animals on day of measurement in g (max.+min.)	No. of unaltered uteri
0	25	0.44 ±0.17	21 (17.4-23.4)	4	20	0.35 ±0.13	26.9 (24.4-30.7)	4
0.5	26	0.89 ±0.4	20.6 (17.4-22.8)	3	15	0.62 ±0.25	26.3 (24.0-28.4)	0
1.5	16	1.29 ±0.4	21.2 (18.8-24.0)	1	15	0.88 ±0.5	27.9 (23.3-31.6)	3
4.5	16	3.14 ±0.58	21.1 (18.5-23.7)	0	15	2.11 ±0.88	27.5 (25.4-32.9)	1
13.5	15	2.93 ±0.69	20.8 (19.4-22.9)	0	15	3.27 ±0.88	26.4 (23.8-29.2)	0
40.5	10	2.76 ±0.89	20.0 (17.1-21.7)	1	10	2.88 ±1.02	28.0 (25.5-31.2)	0

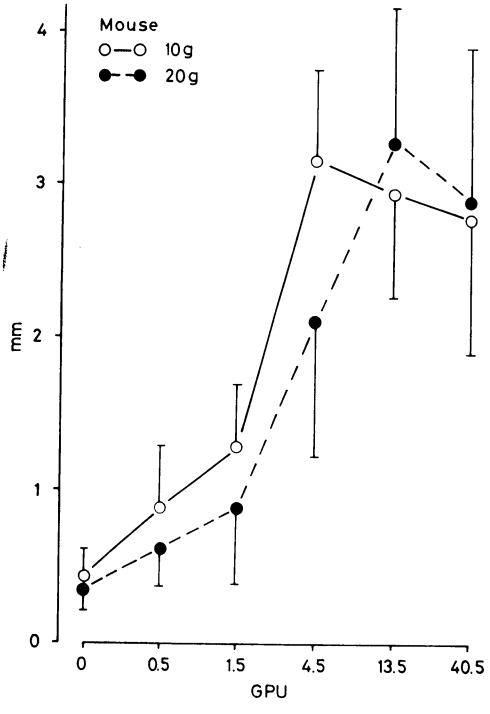

Fig. 1. — Broadening of pubic ligament by relaxin in mice of 10 g and 20 g initial weight after priming with oestradiol benzoate.

Table 1 shows the values of public ligament broadening after treatment with relaxin in mice having an initial weight of 10 g and of 20 g. During the 7 days' priming, the animals increased in weight, in the lower weight class by an average of 10 g and in the higher weight class by about 7 g. Only a small number of animals did not react to priming by an enlarged uterus. There was no close correlation between only slight weight increase and a thread-shaped uterus. Generally, a thread-shaped uterus indicates a smaller broadening of the ligament. However, these latter values were not excluded in calculations of means. In fig. 1 the dose-response curves of broadening of the ligaments in both weight classes of mice are shown graphically. As may be seen, they are S-shaped curves with a relatively steep increase and after reaching the maximum further increase in dosage caused a decrease. Differences between the values of the single dose levels up to the maximal values are statistically significant. Thereafter, because of greater standard deviation, a statistical difference for the decrease in values could not be established.

The P-values here lay just over 0.05. The maximal broadening of the ligament was equal in both groups, lying between 3.1 and 3.3 mm. This was obtained with 4.5 GPU for the lower weight class and with 13.5 GPU for the heavier group. This means that in the lower class, the same broadening was obtained with 1/3 of the dose. In general the greater sensitivity to relaxin in lighter animals is seen in the curve by shifting to the left.

In the second part of the work, priming was attempted with Presomen which provides a broader oestrogen spectrum. Again, mice with an initial weight of 10 g and 20 g were used. Presomen was given in the dosage 2x500 µg and 2x1000 µg. 24 hours after the first dose, relaxin was injected together with the 2nd dose of Presomen. In the 10 g weight class, the dose-response curves were rather flat with the high relaxin doses not reaching an elongation of the pubic ligament of 1 mm. However, in all cases, thickening of the uterus was observed and the weight increase in the mice varied around 2 g on average.

In the class with an initial mouse weight of 20 g after 2x500 µg Presomen priming the curves showed a clear dose-response relationship although with the highest dose, 40.5 GPU not much more than 1 mm increase of ligament breadth occurred. This curve is seen in fig. 2. The differences between zero values and the higher relaxin values were statistically significant with p values over 0.05 using 15 mice in each dose group. In 2 days, the average increase in weight for animals in this group was about 2 g. An oestrogenic effect on the uterus was seen in all cases. By doubling the priming dose to 2x1000 µg Presomen, an almost identical dose response relationship was obtained, i.e. a more marked increase in broadening of the ligament was not obtained.

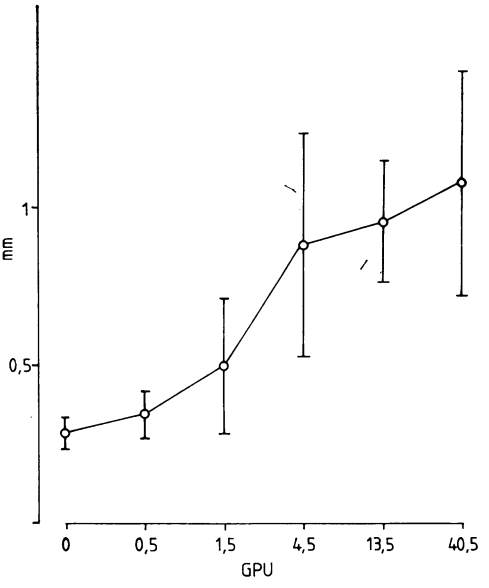

Fig. 2. — Broadening of pubic ligament by relaxin in mice of 20 g initial weight after priming with 2×0,5 mg presomen.

DISCUSSION

The present investigations have shown that the pubic ligament of oestrogen-primed mice reacts by broadening to highly purified relaxin in a dose dependent manner, over a wide range as already described by Steinetz *et al.* using crude substance. After oestradiol benzoate priming, a maximal effect was obtained and thereafter a decrease in the broadening action on the ligament. This effect was not observed by Steinetz and co-workers as they examined only the straight parts of the curves necessary for testing relaxin. Statistical analyses of the decrease after maximal response just failed the limit for significance. Due to lack of relaxin, the groups given the higher doses had to be restricted in numbers.

Comparison of the two weight classes with initial weights of 10 g and 20 g, shows that relaxin testing can also be carried out with lower weight mice, as

already described by Struck ([3]). As Steinetz *et al.* pointed out, a good increase in weight during priming indicates suitability of the ligament for testing. At the end of the 7 day priming period, the weight of the immature mice had practically doubled. The greater sensitivity of the smaller weight group cannot be explained only on account of the higher relationship of relaxin dose to body weight as the weight relation was finally 20:27. Maximal values were obtained in the lower weight group with 4.5 GPU, and with the 7 g heavier mice only with 3 times this dose i.e. 13.5 GPU. The number of mice which did not react to the priming, according to increase in uterine weights, was small. In the animal groups with maximal values, all uteri showed an oestrogen-induced thickening. The few failures in the other groups were included in calculations of mean values and not eliminated as in the work of Steinetz *et al.*

Comparison of the present work with that of Steinetz and coworkers shows that the latter group obtained a broadening of about 2.4 mm already with a dose of 1 GPU, so reaching a higher sensitivity. Whether this is due to the use of another strain of mice, Swiss mice instead of the NMRI strain or to other factors is not known. The time of year in which the investigations are carried out is considered to play a role, the lowest results in pubic broadening being obtained in winter. However, whereas the present work was carried out in winter, the influence of this should be minimal due to the standardized conditions of animal maintenance.

The attempt to achieve quicker priming by using conjugated oestrogen was not successful. With a high dosage of Presomen, a uniform picture concerning thickening of the uterus was obtained as a sign of oestrogenic action. Broadening of the ligament was nevertheless considerably poorer than after 7 days of oestradiol priming. Thus, the observable oestrogen action on the uterus does not necessarily run parallel to the broadening effect on the symphysis. However, even with less broadening of the ligament, well-defined dose-response curves were obtained.

Possibly the weight increase in the mice after treatment with oestrogen is of great importance for a greater broadening effect of relaxin as Steinetz *et al.* have already stated and uterine weight alone is not a guarantee for a good relaxin action.

Concerning the technique of measurement of the pubic ligament it seems advantageous to place the preparation on a slide under the microscope as opposed to the free preparation connected to the lower extremities as done by Steinetz *et al.* Manual stretching by pulling on the hind legs is not necessary and injury of the pubic ligament cannot occur. In addition the geometry is always similar. Also, rapid drying out of the preparation is prevented by protecting with a glass cover.

In conclusion, it can be said that measurement of the pubic ligament still provides a convenient and certain method for estimating an unknown dose of relaxin. However, it should be considered that in the estimation of unknown relaxin concentrations, various doses should be tested to show if the values lie below the maximal range of broadening. When rapid estimation of relaxin is required, the Presomen priming may be used considering that in this way a more qualitative than quantitative estimation is obtainable.

BIBLIOGRAPHY

1) Steinetz B. G., Beach V. L., Kroc R. L., Stasilli N. R., Nussbaum R. E., Nemith P.J., Dun R. K.: *Endocrinology*, *67*, 102, 1960.
2) Schwabe C., Steinetz B. G., Weiss G., Segaloff A., McDonald J. K., O'Byrne E., Hochman J., Carriere B., Goldsmith L.: *Rec. Progr. Horm. Res.*, *34*, 123, 1978.
3) Struck H., Wirkungen und Wirkungsmechanismus des Relaxin. Habilitationsschrift. Medizinische Fakultät der Universität Köln, 1968.